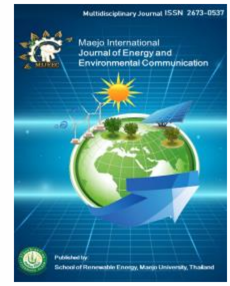
Maejo International Journal of Energy and Environmental Communication 5-2 (2023) 14-20

Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Impact of water temperature on the shipping of berried female broodstock (*Macrobrachium rosenbergii*): A study on survival, egg retention, hatching duration, and hatching success rate

Krasindh Hangsapreurke[1] and Boonyarath Pratoomchat[2*]

[1]Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiangmai, Thailand
[2]Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi, Thailand

ARTICLEINFO

Article history:
Received 02 May 2023
Received in revised form
23 May 2023
Accepted 28 May 2023

Keywords:
Macrobrachium rosenbergii
Water temperature
Broodstock survival
Hatching performance
Water quality parameters

ABSTRACT

This study evaluated the impact of different water temperatures on shipping berried female *Macrobrachium rosenbergii* broodstocks. The broodstocks, averaging 23±0.07 g in weight and 12±0.1 cm in length, were shipped in styrofoam boxes containing 24L of purified freshwater. Three temperature conditions were tested: 28.5±0.5ºC (control), 25.5±0.5ºC (T1), and 21.5±0.5ºC (T2). Various water quality parameters were measured. Mortality rates were significantly lower in T2 (2.60±0.41%) and T1 (8.96±2.09%) compared to the control (24.89±4.96%) ($p<0.05$). The percentage of healthy berried females was higher in T2 (64.18±3.77%) and T1 (56.12±0.59%) than in the control (39.43±2.44%) ($p<0.05$). T2 exhibited the lowest egg weight loss (6.17±0.66 g) and the shortest hatching period (26.33±3.30 hrs) with the highest hatching success rate (90.68±0.34%) ($p<0.05$). Dissolved oxygen levels decreased more in the control group compared to T1 and T2 ($p<0.05$). Total nitrite levels increased significantly in the control group compared to T1 and T2 ($p<0.05$). The study concludes that cooler shipping temperatures, particularly around 21.5ºC, benefit the survival, health, and hatching performance of berried female *M. rosenbergii* broodstocks.

1. Introduction

As global consumer demand increases due to population growth, the aquaculture industry is thriving (Le-Huynh et al., 2022). Central to this success is the giant freshwater prawn, also known as *Macrobrachium rosenbergii*, an important species in aquatic farming. Originating from the diverse ecosystems of the Indo-Pacific, this prawn has transcended its native habitat to become a linchpin in aquaculture endeavors spanning tropical and subtropical regions (Hooper et al., 2023). South and Southeast Asia stand as compelling stages for this narrative, with Thailand and Taiwan rising as virtuoso performers in the art of prawn cultivation. While Asia remains the epicenter of this crustacean's success, its allure has also begun to enchant Caribbean waters. The aquaculture sector in Thailand has long relied on the economic (Hangsapreurke et al., 2020; Tongmee et al., 2020) and ecological contributions of the *M. rosenbergii*, a species integral to both food security and commercial enterprise.

These prawns are uniquely adapted to tropical waterways that merge with brackish waters, enabling a lifecycle that commences in freshwater habitats but relies on saline conditions for key reproductive stages. Despite their significance, detailed scientific guidelines concerning the safe and efficient transport of *M. rosenbergii* broodstocks from farms to hatcheries remain

* Corresponding author.
E-mail address: Boonyara@buu.ac.th (Pratoomchat. B)

conspicuously lacking (Haslawati et al., 2022). The ramifications of this oversight are not merely economic but have far-reaching ecological consequences. The absence of a scientifically validated shipping protocol can result in elevated levels of water-soluble ammonia and other metabolic wastes, causing physiological disruptions ranging from gill damage to compromised immune functions (Spicer et al., 1990). These issues can cascade down the supply chain, affecting larval quality and, ultimately, the sustainability of the prawn population and the industry that relies upon it (Bridges, 2001).

While it is well-established that water temperature during transportation exerts a profound influence on aquatic animal metabolism, including oxygen consumption and respiration rates (Ridgway et al., 2006), the interplay between temperature and other factors like species-specific metabolic rates, size, and stocking density is less well-understood. Prior work on other species, such as the white shrimp (*Penaeus indicus*), has led to the development of bespoke shipping containers that manipulate temperature and oxygen levels to achieve high survival rates (Babu and Marian, 1998). Similarly, research on *Litopenaeus vannamei* post-larvae elucidates how shipping density and temperature interact to affect ammonia levels and, consequently, survival rates. However, these insights have not been systematically applied or validated for *M. rosenbergii*, leaving a significant knowledge gap.

Furthermore, the implications of temperature management extend beyond immediate survival rates, potentially affecting post-transport reproductive capabilities, stress levels, and long-term health of these crucial broodstocks (Khasani et al., 2022). This complexity underscores the urgency of identifying optimal temperature conditions tailored to *M. rosenbergii*'s unique physiology and lifecycle requirements. To address these gaps and complexities, the present study is designed with a dual focus: to empirically investigate the optimal temperature conditions for transporting *M. rosenbergii* broodstocks and to integrate these findings into a holistic, scientifically robust shipping protocol. In so doing, this study aims to not only enhance the economic yield of the Thai aquaculture sector but also to contribute to the broader scientific understanding of stress physiology in aquatic organisms under anthropogenic influences. The long-term vision is to bolster the resilience and sustainability of the giant freshwater prawn industry, providing a template that could be adapted for other aquaculture species and ecosystems.

2. Material and methods

2.1 Sample preparation

The healthy berried (*M. rosenbergii* broodstocks) females (egg at heart beating stage) at an average weight of 23±0.07 g and 12±0.1 cm in total length, approximately 25 kg from a private farm in Chonburi Province, Thailand, were prepared and acclimated in 500 L fiberglass tank for a day before starting the experiment.

2.2 Styrofoam box preparation for shipping

Styrofoam boxes with lids at 42.5 X 57.5 X 30 cm^3 containing 24 L of fresh water were used. Box lids were drilled for inserting the aeration tube and thermometer. The plastic ice packs were prepared for temperature control. Each experimental shipping box was overlaid on PVC pipes in 2.5 cm diameter for shaking as transport simulation.

2.3 Experimental design

The two treatments and control of this study followed the three temperatures of shipping medium as 28.5 ± 0.5^{o} C (control) 25.5 ± 0.5^{o}C (T1) and 21.5 ± 0.5^{o}C (T2) using a completely randomized design (CRD). In addition, the three replications of each treatment and control were done. The experimental berried females were randomly stocked into styrofoam boxes with a total weight of 2.4 kg/box containing 24L of cleaned fresh water and put the ice pack into the shipping boxes to control the temperature according to the experimental design throughout the experiment. All shipping boxes were shaken by moving the PVC pipe laid beneath the box as in the actual situation.

The shipping medium inside the boxes was sampled at the beginning and the end for dissolved oxygen, total ammonia, and nitrite. Total ammonia and nitrite were analyzed by the method of Hansen and Grasshoff (1983). Dissolved oxygen was detected by D.O. meter YSI model 85/10FT. After 12 hours of shipping test, all ripped females were checked for mortality and healthiness by visual assessment using their carried eggs' fullness and response. The removal eggs were collected and filtrated through the screen net and weighed using an electrical balance. The study framework and relevant materials are outlined in Figure 1.

Figure 1. Experimental framework and culturing procedure: A) broodstocks growing, B) broodstocks inside culture box, C) harvesting, D) measuring the length of induvial, E) broodstocks with eggs, and F) broodstocks eggs release

2.4 Hatching rate and hatching period

After the shipping test was finished, the three ripped females from each replication of treatments and control were randomly sampled to a 250 L hatching tank containing 15ppt cleaned water at ambient temperature, respectively. The ripped females in each tank were individually tagged and weighed (initial weight). The three replications of each treatment and control were done.

Observation them every four hours until totally hatched, the hatching period was recorded, and then the female without eggs was individually weighed (final weight). The individual lost weight as egg weight was calculated by initial weight–final weight. The number of eggs from the individual female was the lost weight divided by 0.00013027 (average individual egg weight in grams). After that, the larval number from each tank was counted and further calculated for the hatching rate.

2.5 Data analysis

The percentage of healthy ripped females, mortality rate, removal eggs weight, hatching rate, hatching period, the increasing values of total ammonium and total nitrite, and the decreasing value of dissolved oxygen were respectively analyzed by one-way ANOVA for statistical differences and compared between groups by Duncan's New multiple range test using SPSS for windows version 11.5 license code 30025 36098 54100 85475 59009 9625.

In the experimental process, fermentation took place in tanks larger than 6 liters, fitted with an airlock and silicone stopper. Straining was achieved using muslin or filter fabric. A hydrometer or refractometer measured sugar and alcohol content, while temperatures were monitored with a thermometer. The final product was bottled and sealed with corkscrews.

3. Results and discussion

3.1 Ripped berried female

The production of postlarvae for *M. rosenbergii*, commonly known as the giant freshwater prawn, relies on a consistent supply of females carrying eggs. In tropical climates, hatcheries often have the advantage of sourcing these egg-bearing females year-round either from designated culture ponds or natural water bodies like rivers, estuaries, and lakes where the species is native (Islam et al., 2023). However, in temperate and subtropical zones, the standard practice involves the collection of mature males and females during autumn and keeping them in indoor facilities with temperature regulation throughout the winter months. The physical characteristics of *M. rosenbergii* make it distinguishable. The prawn can achieve a length exceeding 30 centimeters or approximately 12 inches. While the dominant hue is a shade of brown, the color can differ, especially among the younger prawns, which can exhibit a greenish tint and subtle vertical lines (Yasa et al., 2019).

A noticeable feature is the prawn's rostrum, outfitted with 11 to 14 teeth on its dorsal side and 8 to 11 on its ventral side. One of the most unique features of *M. rosenbergii* is its set of walking legs. The first pair are slender and elongated, ending in fine claws that serve as feeding appendages. The second pair, in contrast, are considerably more robust. What sets these larger claws apart are the thick bristles that envelop them, lending a soft, velvety texture (Borisov et al., 2022). Females possess a broader abdominal region and their second pair of walking legs, also known as pereiopods, are smaller in size compared to those of males. Gender can also be determined by the location of the genital openings; for females, they are situated on the segment associated with the fifth pair of walking legs, while for males, these openings are found on the segment linked to the third (Aquino et al., 2022). After 12 hours of shipping test, the results showed that the mortality rate of the control group was significantly ($p<0.05$), higher than those of T1 (25.5±0.5°C) and T2 (21.5±0.5°C). The percentage of healthy ripped berried females showed the significant highest ($p<0.05$) in T2. The average weight of removed eggs in T2 was significantly ($p<0.05$) lower than those of T1 and control ($p<0.05$). Furthermore, the hatching period and hatching rate of T2 were significantly ($p<0.05$) lower and higher than the others, respectively (Table 1).

Table 1. Mortality rate, percentage of healthy berried female, removal egg weight, hatching period and hatching rate of berried female after 12 hours of shipping

Parameter	**Control**	**T1**	**T2**
	28.5±0.5°C	25.5±0.5°C	21.5±0.5°C
Mortality rate (%)	$24.89±4.96^{a}$	$8.96±2.09^{b}$	$2.60±0.41^{b}$
Healthy female (%)	$39.43±2.44^{c}$	$56.12±0.59^{b}$	$64.18±3.77^{a}$
Eggs removal (g)	$10.39±0.73^{a}$	$9.91±0.82^{a}$	$6.17±0.66^{b}$
Hatching time (hrs)	$69.33±8.67^{a}$	$43.33±8.67^{b}$	$26.33±3.30^{c}$
Hatching rate (%)	$39.67±2.61^{c}$	$72.89±3.42^{b}$	$90.68±0.34^{a}$

Note: Data represented as Mean±S.E. Value in the same row with different superscripts are significantly different ($p<0.05$).

The mortality rate of ripped berried females in shipping medium at 21.5±0.5°C (T2) was the significant lowest and significantly ($p<0.05$) higher than a healthy number. The number of removal eggs of T2 was also significantly ($p<0.05$) lower than the others. Furthermore, the hatching period and hatching rate of T2 showed the significant ($p<0.05$) shortest period and the highest percentage, respectively. This is a result of better water quality when compared to the others. The results showed the significant decrease of dissolved oxygen cooperated with a dramatic increase in nitrite in the shipping medium of control (28.5±0.5°C) and T1 (25.5±0.5°C). Dissolved oxygen of the shipping medium decreased approximately 3.4 times and 1.9 times, respectively when compared to the value of T2 while the increasing value of nitrite in the control group showed double values when compared to T2.

3.2 Water quality

Over time, the approach to shrimp farming has transitioned from extensive methods to more intensive systems, which require increased inputs of high-quality feed and a reliable water supply. In these conventional intensive systems, water quality, which can degrade over time, is often managed by replacing old water with fresh, externally sourced water to ensure optimal conditions for shrimp growth (Tao et al., 2021). However, the farming of *M. rosenbergii* has caught the attention of both researchers and agricultural practitioners, not just because of its market demand and flavor profile, but also because its cultivation appears to be both economically feasible and environmentally less damaging compared to traditional shrimp farming (Fadhilah et al., 2019).

There is existing information on the stocking density and specific needs of *M. rosenbergii* when raised in monoculture setups (Kunda et al., 2008). Yet, maximizing the efficiency of freshwater prawn farming still necessitates the identification and evaluation of needs such as the diet and nutrients of the species being farmed. Several factors contribute to the successful rearing of a given species, including the chosen technology, location, infrastructure, and expertise in production management, among others (Sahoo et al., 2006). In aquaculture settings that employ water recycling methods, maintaining water quality is of paramount importance. Ensuring that the water quality stays within optimal ranges is crucial for promoting the health and rapid growth of the aquatic species being farmed. The results found that the decrease of dissolved oxygen in the control group was significantly (p<0.05) higher than those of T1 and T2 and the value of T1 was also significantly (p<0.05) higher than that of T2 (Table 2).

Table 2. Dissolved oxygen at 0 h and 12 h of shipping period and decreasing values.

Shipping period Treatment	Dissolved oxygen (mg/l)		
	0 hrs	**12 hrs**	**Decreased value**
Control (28.5±0.5ºC)	5.67±0.02	3.38±0.06^{b}	2.29±0.05^{a}
T1 (25.5±0.5ºC)	5.84±0.04	4.14±0.16^{a}	1.32±0.18^{b}
T2 (21.5±0.5ºC)	5.62±0.10	4.49±0.02^{a}	0.68±0.10^{c}

Note: Data represented as Mean±S.E. Value in the same column with different superscripts are significantly different (p<0.05).

The ammonia concentration from all treatments was increased, but their decreasing values were not significantly different (p<0.05). However, the increasing value of nitrite in the control group was significantly (p<0.05) higher than those of T1 and T2, and the value of T1 was also significantly (p<0.05) higher than that of T2 (Table 3). Thus, shipping at a higher temperature would have a higher protein metabolism and produce higher ammonia and nitrite. Therefore, the quantity of ammonia and nitrite in control at ambient temperature and 25.5+0.5ºC was higher than that of 21.5+0.5ºC, which corresponds with a report by Chen and Chia (1996), who reported that ammonia excretion of juvenile *Scylla serrata* would increase significantly, while temperature increased, and salinity decreased and concluded that nitrogen metabolism was depended on temperature and salinity by transforming urea to ammonia. In the similar with *P. japonicus* in salinity 37 and 21 ppt. and temperatures 10-14°C and 25°C, this research indicated the higher excretion of ammonia depended on high temperature and low salinity (Farhadi et al., 2022).

This experiment found that nitrite and DO decreasing values in control and T1 were higher than that of T2 (p<0.05), while the ammonia values were not different among groups. This indicated that the higher metabolism in shipping at ambient temperature and 25.5+0.5ºC were higher than 21.5+0.5ºC. So, shipping at 21.5+0.5ºC is less in toxicity from nitrite and still high dissolved oxygen, which is declared to improve broodstock's quality due to higher survival rate, higher healthy number, and superior hatching performance. The study concurred with Xiaotao et al. (1999), who have demonstrated that the oxygen consumption rate, energy consumption and waste excretion rate of CO_2 and NH_3-N in juvenile *M. rosenbergii* were the lowest at 20°C (the lowest temperature) among 20, 24, 28 and 32°C.

This is also similar to Yang et al. (2012), who had been the transport of juvenile marketable prawns (*M. rosenbergii*) at a stocking density of 160 kg ton1 water in each barrel for trips lasting over 10 h, a 50% water exchange should be made at least once midway in the trip, maintain the temperature 18–19°C, and add sea salt to adjust the salinity to 2–3 ppt with 90% or higher for survival rate. Environmental temperature profoundly affects the physiology of all poikilotherms, including freshwater prawn (*M. rosenbergii*); when the water temperature is lowered, the prawn’s oxygen consumption rate and waste excretion rate are also reduced (Yang et al., 2012).

Table 3. Total ammonia, total nitrite at 0 h and 12 h of shipping period and decreasing values

Shipping period Treatment	NH_3-N (mg/l)			NO_2-N (mg/l)		
	0 h	**12 h**	**Increased value**	**0 h**	**12 h**	**Increased value**
Control (28.5±0.5ºC)	0.31±0.06	6.79±0.44	6.48±0.40	0.00±0.00	0.13±0.01^{a}	0.13±0.00^{a}
T1 (25.5±0.5ºC)	0.36±0.07	6.47±0.54	6.11±0.30	0.01±0.01	0.09±0.02^{ab}	0.08±0.01^{ab}
T2 (21.5±0.5ºC)	0.29±0.18	6.05±0.18	5.76±0.50	0.00±0.00	0.06±0.01^{b}	0.06±0.01^{b}

Note: Data represented as Mean±S.E. Value in the same column with different superscripts are significantly different (p<0.05).

The experiments of Manush et al. (2004), Salin (2005), and Sun (2009) revealed that lowering the water temperature gradually can significantly improve the survival rate of *M. rosenbergii* transportation. A former report revealed that toxicity from nitrite would reduce the oxygen exchangeability of *Litopenaeus vannamei*, especially in lower salinity cultures. This phenomenon was nitrite made a stable oxidized form of hemocyanin, which could not release oxygen to the tissues, produced some inherited abnormalities (Boyd, 1990). It is known that the development of anaerobic metabolism leads to the production of L-lactate and acidic pH (Whiteley and Taylor, 1992; Jackson et al., 2001; Ridgway et al., 2006).

Anaerobic metabolism can be elicited by a hypoxic environment and by an increase in the metabolic rate of animals due to an increase in temperature, which boosts the energy demands of the animal (Durand et al., 2000; Ridgway et al., 2006). The glucose concentration increment is related to the mobilization of energy stores upon requirement during stressful conditions in lower O_2 availability as a source of fuel for anaerobic metabolism resulting in lactate production. Lobster metabolism supplemented energy cell requirements by anaerobic metabolism, which was indicated by a rapid lactate accumulation. Lactate was the major end-product of anaerobic metabolism in Crustacea (Spicer et al., 1990). Therefore, the increase of lactate concentration in the hemolymph during aerial exposure indicates anaerobic metabolism and presumably comes from the inability to maintain an adequate supply of oxygen to tissues (Spicer et al., 1990; Ridgway et al., 2006).

Moreover, lactate acts as a positive effector in causing an increase in hemocyanin oxygen affinity (Bridges, 2001). Furthermore, the other report on the commercial transport of Homarus americanus and the recovery process in water specifically at different animal body temperatures (6 and 15 °C) and air exposure found that blood glucose, lactate, total protein, and cholesterol were significantly higher in the group with high body temperature compared to those with low temperature until 96 h after immersion in the recovery tank (Lorenzon et al., 2007). Thus, this exploration confirmed and supported that shipping at optimal low temperatures will bring the shipping quality of *M. rosenbergii* broodstock.

3.3 Forging a sustainable future through M. rosenbergii farming

Add titeIn the realm of sustainable aquaculture, the interaction between water temperature and the shipping of berried female broodstock (*M. rosenbergii*) has garnered attention due to its profound implications for survival rates, egg retention, hatching duration, and hatching success rates (Mohamad et al., 2018). A comprehensive study delving into this relationship sheds light on its multifaceted dynamics. The concept of a biocircular green economy (BCG) provides a robust framework for contextualizing the significance of farming the giant freshwater prawn, *M. rosenbergii*. This species aligns harmoniously with the principles of BCG for several compelling reasons. Its intrinsic efficiency in terms of accelerated growth and commendable survival rates resonates with the core tenet of resource optimization, exemplifying the first pillar of the BCG model. By curbing wastage and enhancing yield sustainability, this alignment underpins economic and environmental viability (Pimpimol et al., 2020).

Moreover, the environmental impact of prawn farming can be mitigated by transitioning to plant-based feeds. Departing from the ecologically taxing tradition of fish meal utilization, the adaptability of *M. rosenbergii* to plant-centric diets heralds a shift towards sustainable feed alternatives. This pivotal shift aligns with the second pillar of BCG, spotlighting the imperative to reduce environmental burdens. The facets of a circular economy further embellish this paradigm. Harnessing the waste by-products from prawn farming as fertilizers or for biogas production manifests the cyclical principles of resource circulation, seamlessly connecting with the third pillar of BCG (Tongmee et al., 2021). This symbiosis with circularity not only redefines waste but also mirrors the ecosystem's intricate interconnectedness.

Technological innovation also finds a realm in this tapestry. The requisites of water quality and feed management within prawn farming serve as fertile ground for pioneering technological solutions. This manifestation of green technologies underscores the BCG principle of integrating innovation into economic activities, paving the way for a synergistic evolution. Embarking on a broader canvas, the congruence between the farming of *M. rosenbergii* and the United Nations Sustainable Development Goals (SDGs) is striking. Beyond economic gains, the creation of job opportunities in rural spheres aligns with SDG 1 (No Poverty) and SDG 8 (Decent Work and Economic Growth). The promise of substantial yields resonates with SDG 2 (Zero Hunger), offering a conduit for protein-rich sustenance and bolstering food security (Deshpande, and Aspen (2018). The potential to fine-tune prawn feed to encompass essential nutrients harmonizes with SDG 3 (Good Health and Well-being), echoing the pursuit of holistic nourishment.

By virtue of demanding water management, prawn farming indirectly champions SDG 6 (Clean Water and Sanitation), fostering conscientious water stewardship. Aligned with eco-consciousness, sustainable prawn farming abides by SDG 14 (Life Below Water), striving to coexist harmoniously with aquatic ecosystems (Schuhbauer et al., 2020). The collaborative orchestration required for these achievements reverberates with the essence of SDG 17 (Partnerships for the Goals), accentuating the necessity for cross-sectoral cooperation. In summation, the judicious amalgamation of planning, management, and foresight in the farming of *M. rosenbergii* projects a paradigm for sustainable aquaculture, inherently intertwined with the ethos of the Bio Circular Green Economy. This harmonization serves as a lodestar for economic prosperity, environmental stewardship, and the attainment of diverse Sustainable Development Goals.

4. Conclusion

The impact of water temperature on the shipping of berried female broodstock of *M. rosenbergii* has been examined meticulously in this study, producing insightful outcomes that are essential for the sustainable and economically viable aquaculture of tropical freshwater prawns. As prawn farming emerges as a sustainable alternative to shrimp farming, particularly in areas where costly coastal sites are not necessary, understanding the parameters that can improve survival and reproductive success becomes increasingly important. This study has identified an optimal water temperature of 21.5 ± 0.5°C for a 12-hour shipping duration for *M. rosenbergii* broodstock. At this specific temperature range, the study found the highest survival rates and the greatest proportion of healthy broodstock. Additionally, this temperature minimized stress factors such as oxygen consumption, ammonia, and nitrite excretion. The optimal water temperature also resulted in the highest hatching success rate, proving its efficacy not only in survival but also in subsequent generations, thereby enhancing the overall productivity and economic efficiency of prawn farming operations. By reducing stress and waste excretion, it minimizes the need for water treatment and lowers pollution risk, aligning with sustainability goals. Given these significant findings, it's crucial for industry stakeholders to integrate these temperature guidelines into shipping protocols for enhanced sustainability and long-term success in prawn aquaculture.

Conflict of Interest Declaration

The authors assert that there are no conflicts or personal affiliations that might be construed as impacting the outcomes shared in this research.

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